CURRENT STATUS OF IMAGING MICROBIAL BIOFILMS IN THREE-DIMENSIONAL OPAQUE POROUS MEDIA USING X-RAY MICROTOMOGRAPHY

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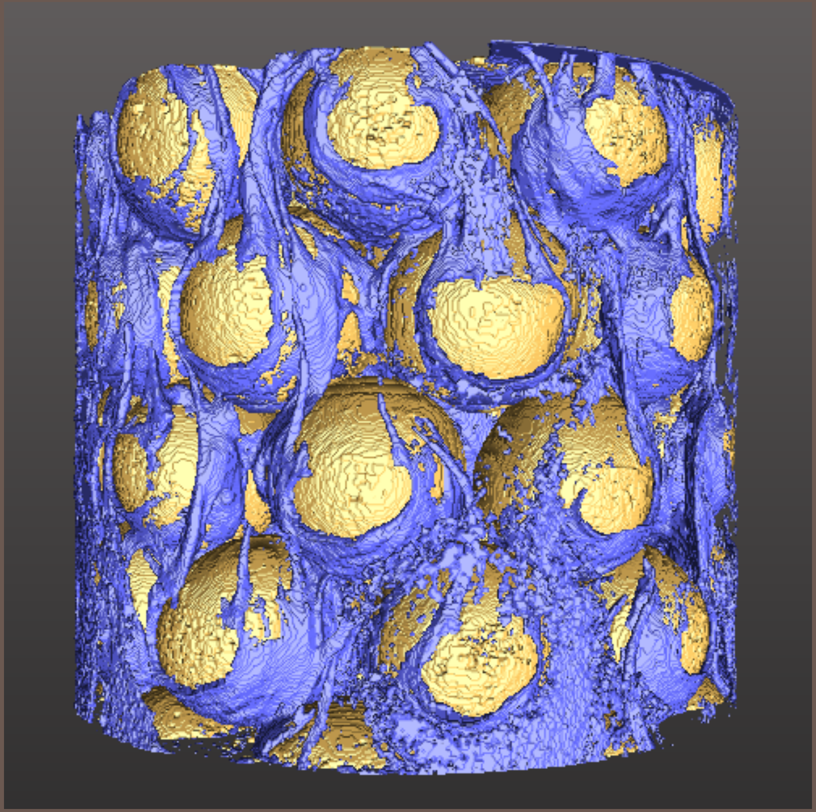
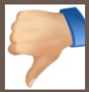


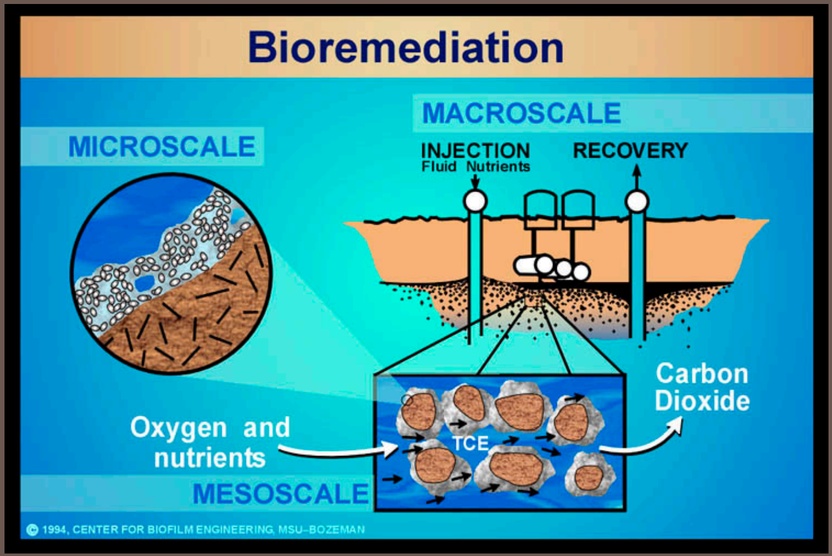
Biofilms and Biofilm Architecture

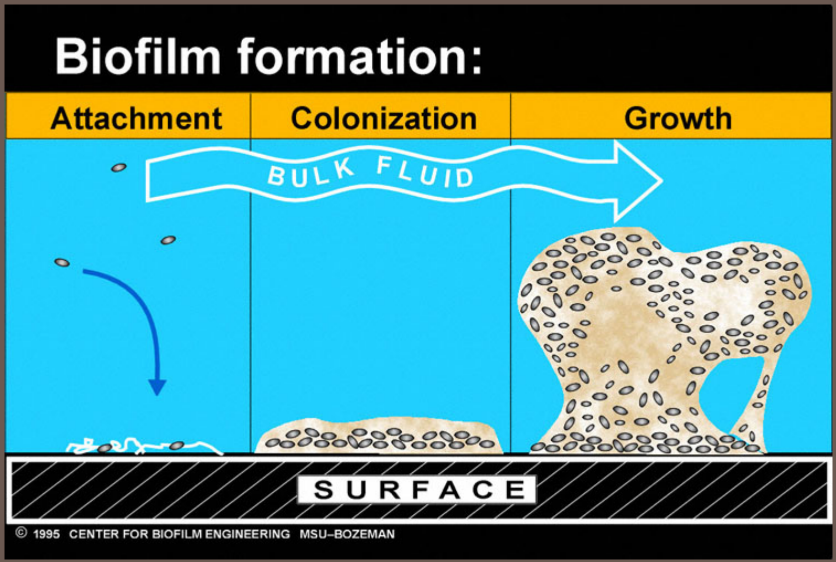
- Soils and rocks – and engineering

  - Bioclogging

  - Bioremediation

  - Microbial enhanced oil recovery

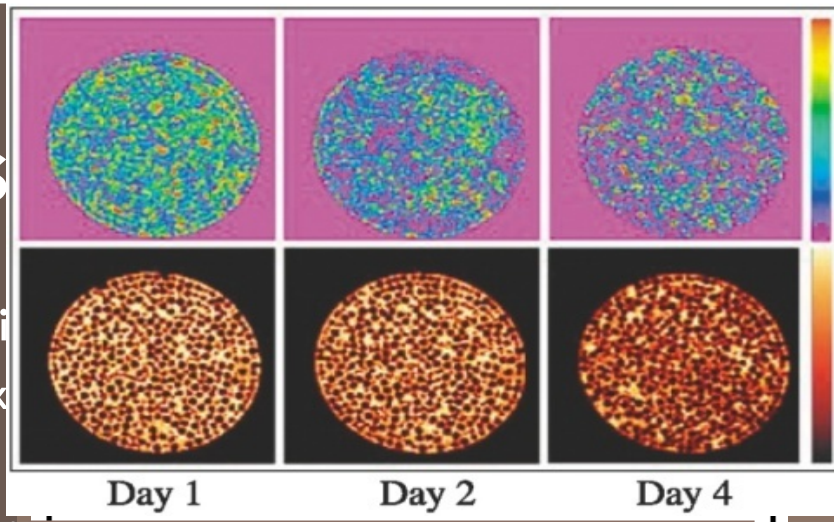
- We image:
  - Biofilm surface morphology in a porous medium
  - Not internal geometry and/or individual cells
  - Spatial (and temporal) arrangement of biofilm in 3D porous media





*Images courtesy of the Center for Biofilm Engineering, Montana State University

Research Objectives

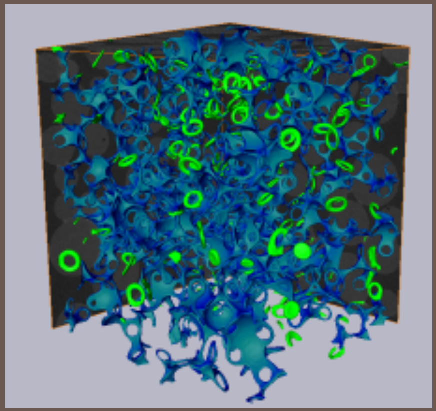


Seymour et al. 2004

- To measure 3D architecture of biofilms i
  (soft object embedded in a hard matrix
  - Past approaches:
    - Destructive methods such as thin-sectioni
    - Two-dimensional micromodel systems (e.g. ... 2002)
    - Numerous studies of 3D growth on flat substrate (no 3D porous medium) using CLSM – or on single grain only
    - Magnetic Resonance Microscopy/Nuclear Magnetic Resonance (e.g. *Seymour et al.*, 2004) – limited resolution and long acquisition times
    - Nano-scale x-ray tomography observations of the biomass only (minus porous medium) (e.g. *Thieme et al.*, 2003)
    - 3D studies using CLSM on index-matched media (Leis et al., 2006)
- To provide 3D biofilm geometries for validation of existing theory and numerical models:
- Ex.:
  - Individual-based Models (IbM), e.g. *Kreft et al.*, 1998, 2001
  - IbM with lattice-Boltzmann model (*Graf von der Schulenburg et al.*, 2009)

Why x-ray tomography?

- Simple answer: Three-dimensional information in opaque porous systems!
- Fast (~5-10 mins per scan)
- High resolution (~5 μm)
- No need to index-match
- "Non-destructive"
  - (to the porous medium)



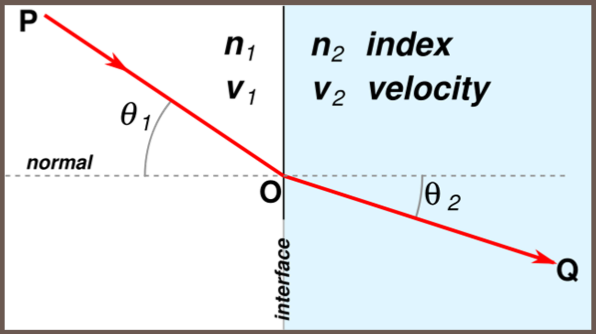
Porter & Wildenschild (2009)

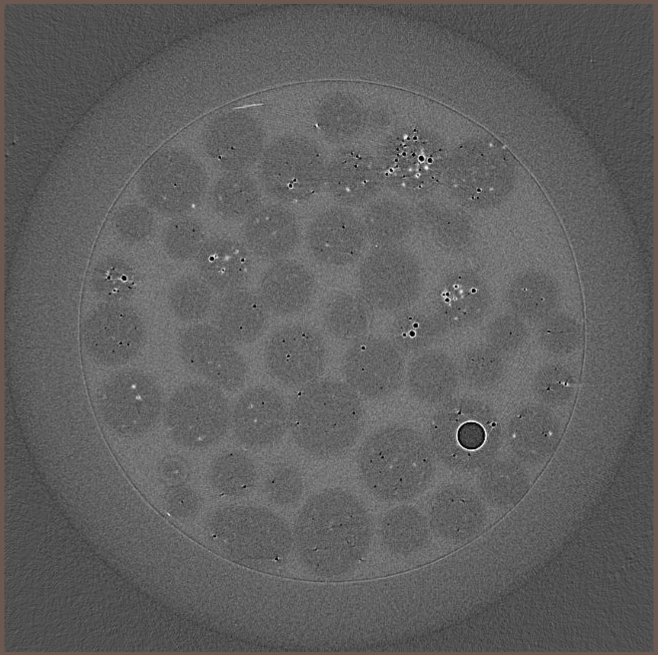
- Potential to quantify:
  - structural arrangement
  - feedback with permeability/hydrodynamics
  - growth patterns/rates
  - mass transfer rates

- flow conditions
- growth conditions
- microbial species

Phase contrast tomography

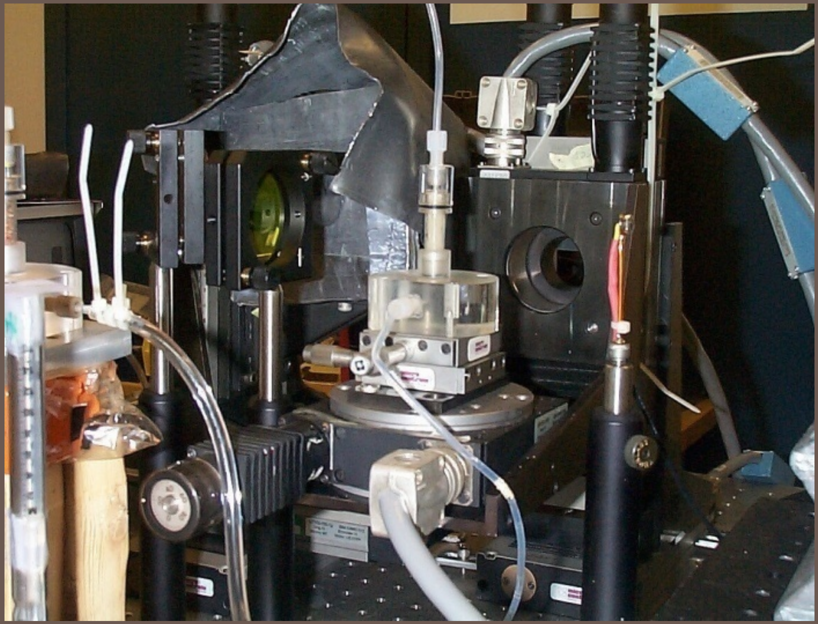
- Based on differences in refractive index (Snell's Law)
- Index-matching used with CLSM
- Can be used with x-rays as well
  - Momose et al. (1995, 1996) - medical imaging
- Work at Swiss Light Course (TOMCAT beam-line) and XRT Ltd. in Melbourne, Australia
- Relatively small variations in refractive index between water and biofilm





Horizontal slice of biomasss? in polystyrene bead pack

Computed X-ray Microtomography (CMT)



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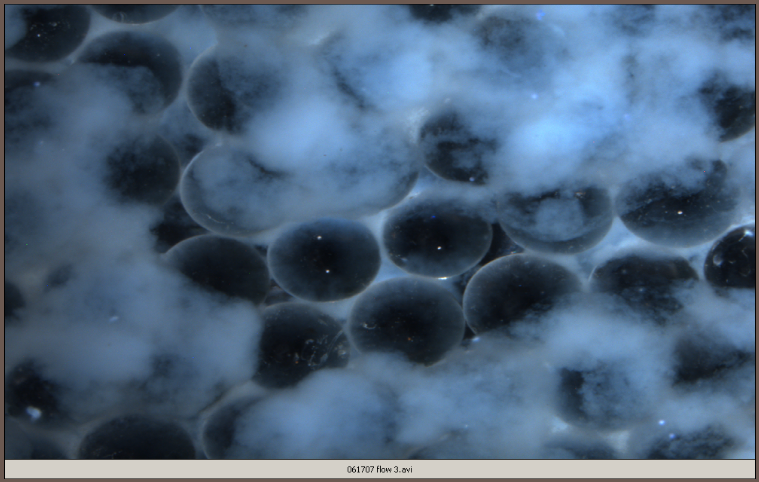
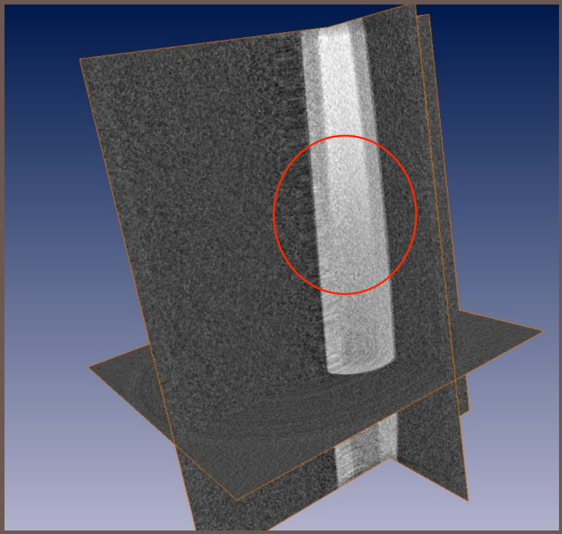
Limitations of CMT for Imaging Biofilm

- X-ray cross-section of a biofilm is similar to that of water –> need contrast agent
- Conventional contrast agents, e.g. potassium iodide, commercial medical contrast solutions (Fenestra, Isovue) diffuse readily into the biofilm
- X-ray exposure is expected to kill or severely inhibit biofilm growth
- Access to synchrotron x-ray sources (monochromatic radiation) is advantageous, but <u>not unlimited</u>
- Commercial micro CT systems do not support specific photoelectric edge-enhanced delineation



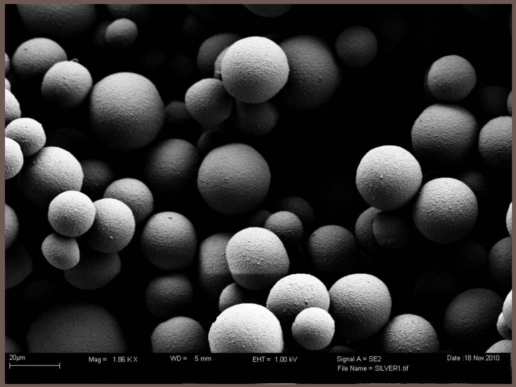


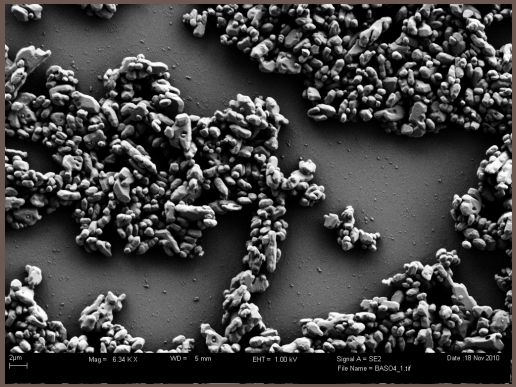
Diffuse interface

X-ray CMT Contrast Agents

- To date, two particle-based contrast agents have been used for imaging biofilms in porous media:

- 1. Silver-coated hollow glass microspheres
  - *Iltis et al.* (2011), *Water Resources Research*
- 2. Barium sulfate suspension
  - *Davit et al.* (2010), *Journal of Microscopy*



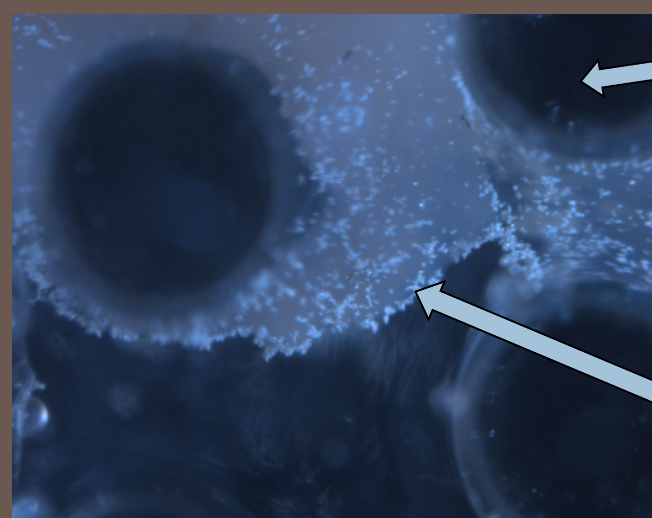


(Adrienne Phillips and James Connolly, MSU, 2010)

1. Silver Microsphere Contrast Agent

- Silver coated microspheres
  - ~10 μm diameter
  - Neutrally buoyant
  - High attenuation via Ag absorption edge at ~ 26 keV
- Could use a variety of elemental contrast agents (w. photoelectric edges between ~ 15-40 keV such as Rb, Sr, Pd, Ag, I, Cs, Ba….)

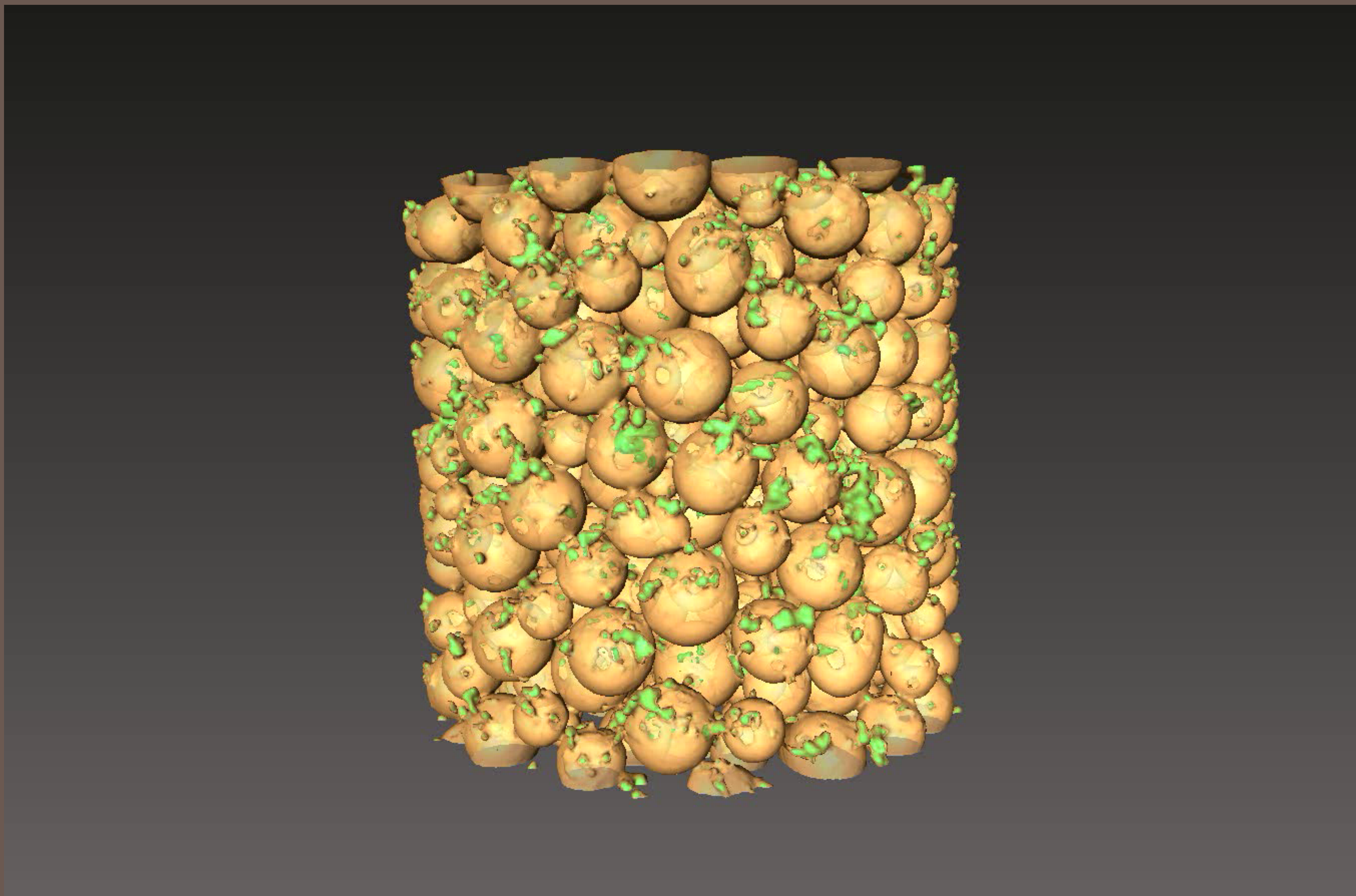
Biofilm



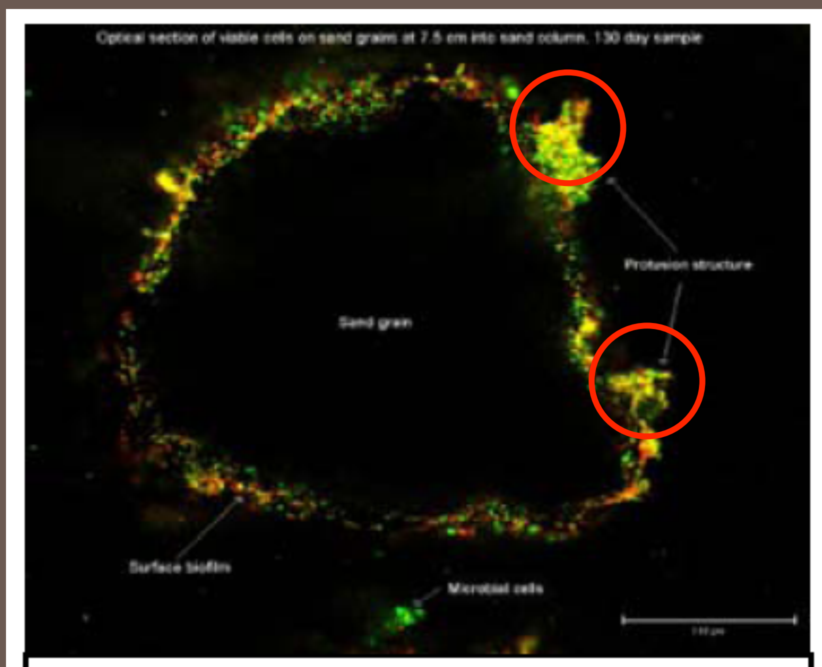
Flow cell column

Biofilm-attached silver microspheres

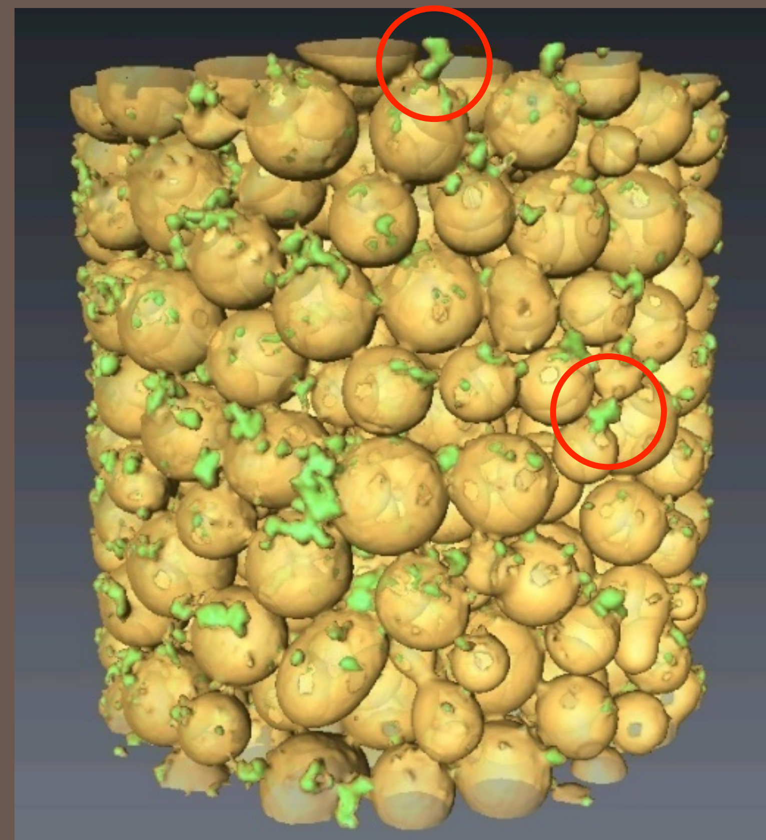
3D Biofilm Geometry



3D Biofilm Geometry

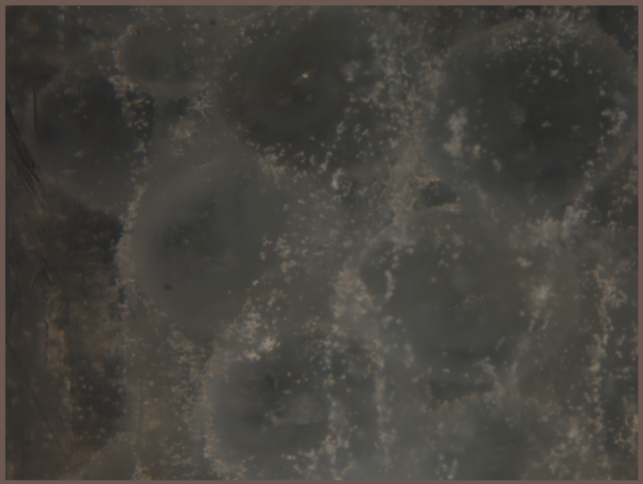


Rodriguez and Bishop (2007)



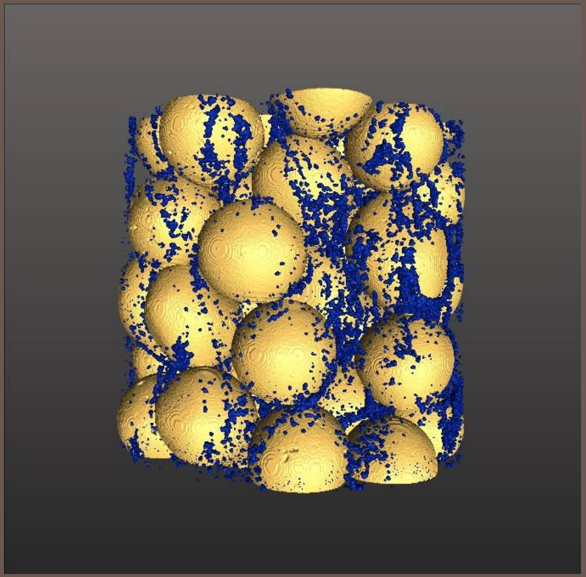
Shewanella oneidensis biofilm in glass bead pack (*Iltis et al.*, 2011)

Segmentation and point-wrap algorithm (delineation of biofilm)

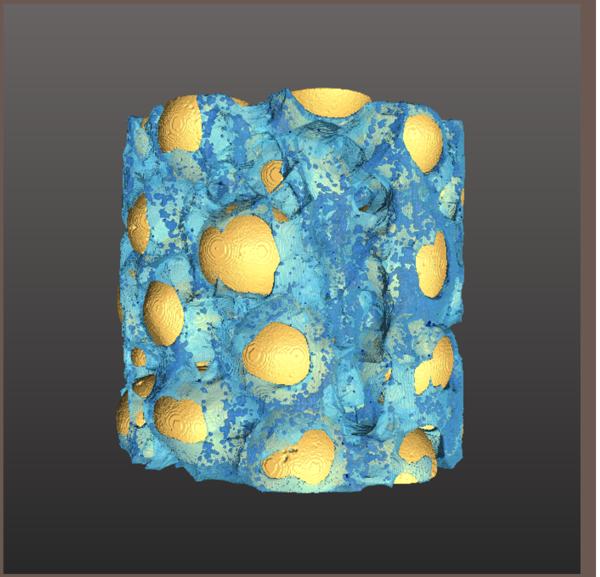


Biofilm with silver microspheres attached in glass bead pack

- light microscopy



Glass bead pack (yellow) with biofilm-associated silver particles (blue)



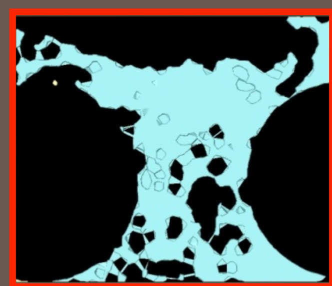
(Avizo Fire®) PointWrap representation of biofilm

2D Biofilm Analysis – Ag particle approach

CMT

Light microscopy

Feature A



Feature B

	Surface Extraction		Point Wrap	
	Surface Area	Diff.	Surface Area	Diff.
	mm^2	%	mm^2	%
Feature A: LM	0.521	0	0.797	0
Feature A: CMT	0.495	-4.91	0.765	-4.13
Feature B: LM	0.639	0	0.999	0
Feature B: CMT	0.648	+1.01	1.017	+1.82

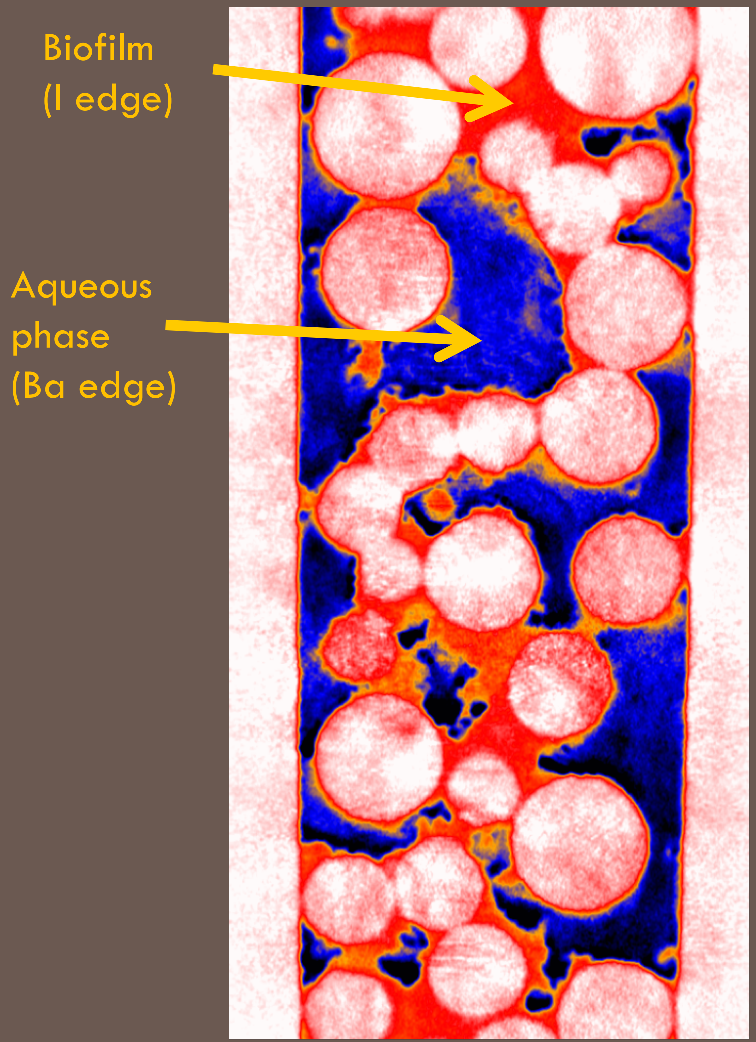
Additional details on our companion poster (tonight) – and in *Iltis et al. (2011)*

2. Barium Sulfate Contrast Agent

- Commercially available, medical-grade suspension
- ~ 1μm sized particles
- Substitutes for aqueous phase while size-excluded from the biofilm phase
- Biofilm phase doped with KI

- *Davit et al.* (2010) used conventional CMT system

- Synchrotron:
  - Aqueous phase identified via barium absorption edge at ~ 37.5 keV
  - Biofilm phase via iodine edge at ~33.5 keV
  - Image subtraction – requires highly accurate registration

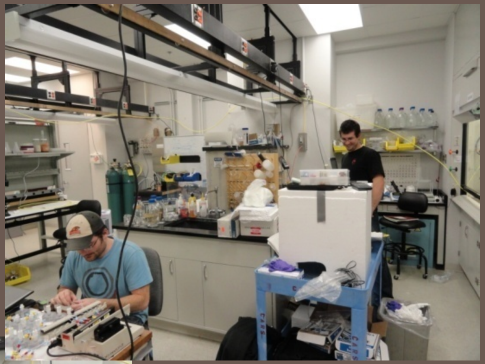


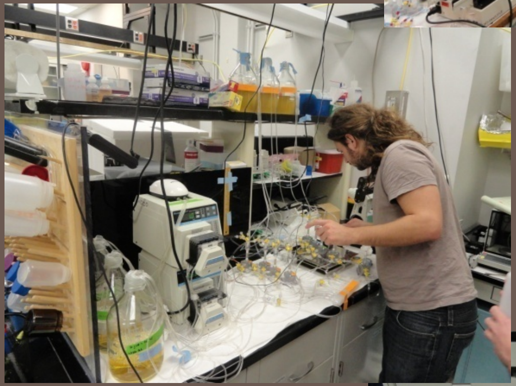
Davit et al. (2010)

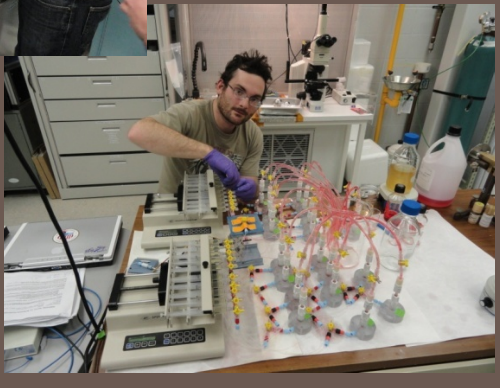
Contrast Agent Comparison in 2D and 3D:

Gabe Iltis, Ryan Armstrong, Yohan Davit, and James Connelly

- Biofilm imaging involves “living” at the beam-line while the biofilm grows
- Compare microsphere and suspension approach in both 2D micromodels and 3D flow columns
- 2D: CMT vs. CLSM
- Data for Ag and $BaSO_4$ for same systems
- Results in enormous amounts of data - and image processing tedium..



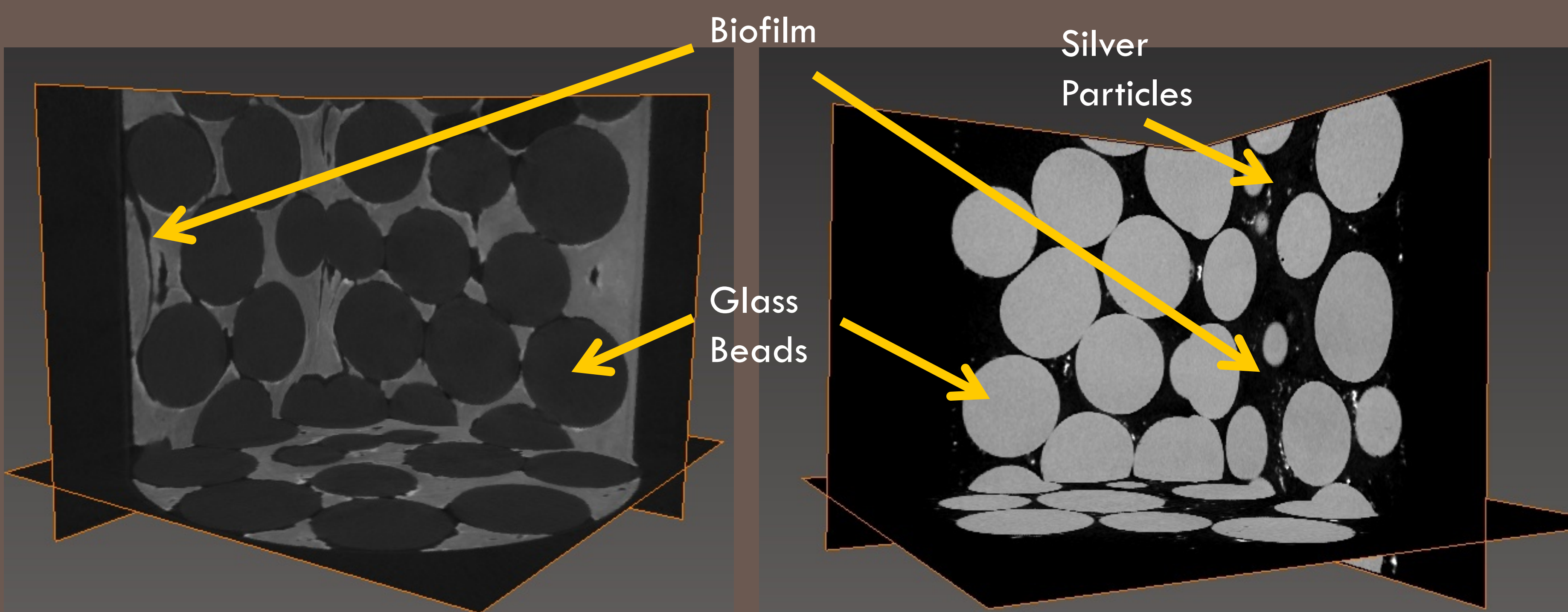




Contrast Agent Comparison Gray Scale Data

Barium sulfate

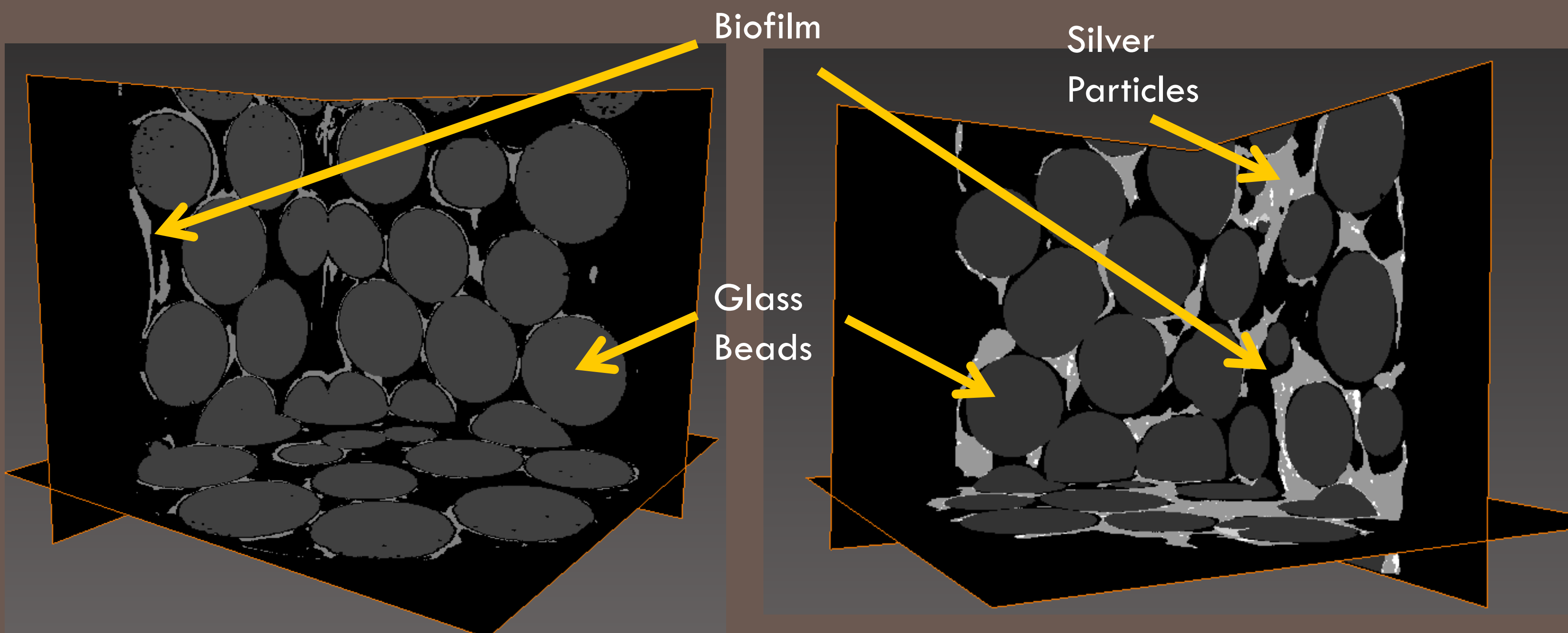
Silver-coated microspheres



Contrast Agent Comparison: Segmentation

Barium sulfate

Silver-coated microspheres



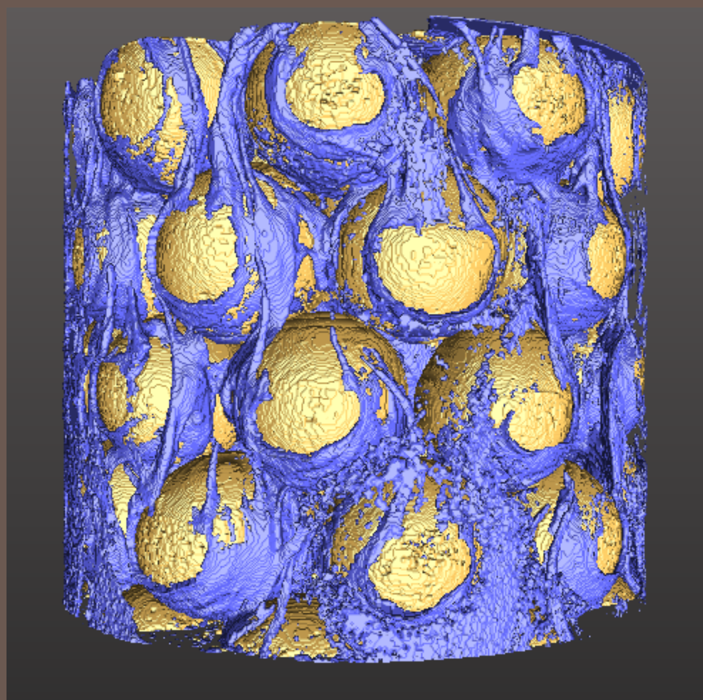
Aqueous phase disappears

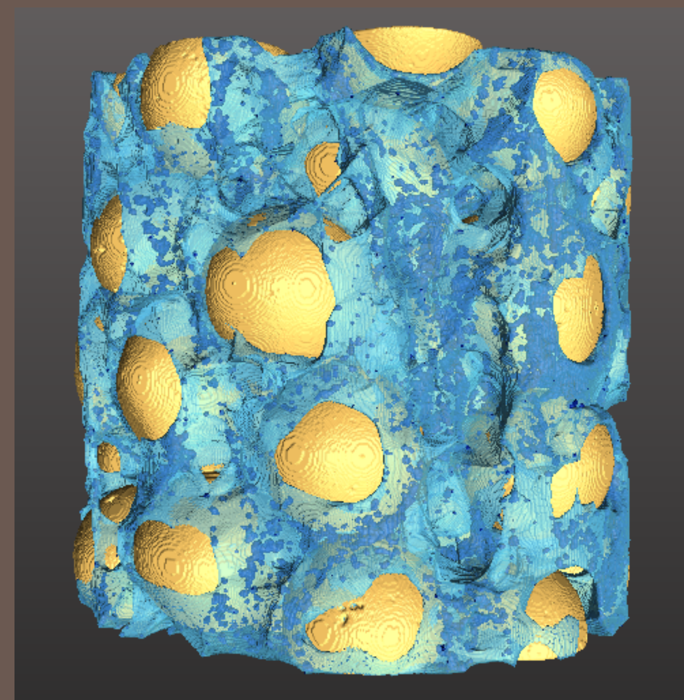
Biofilm delineated by Ag particles

Contrast Ag  n:
3-D Renderi

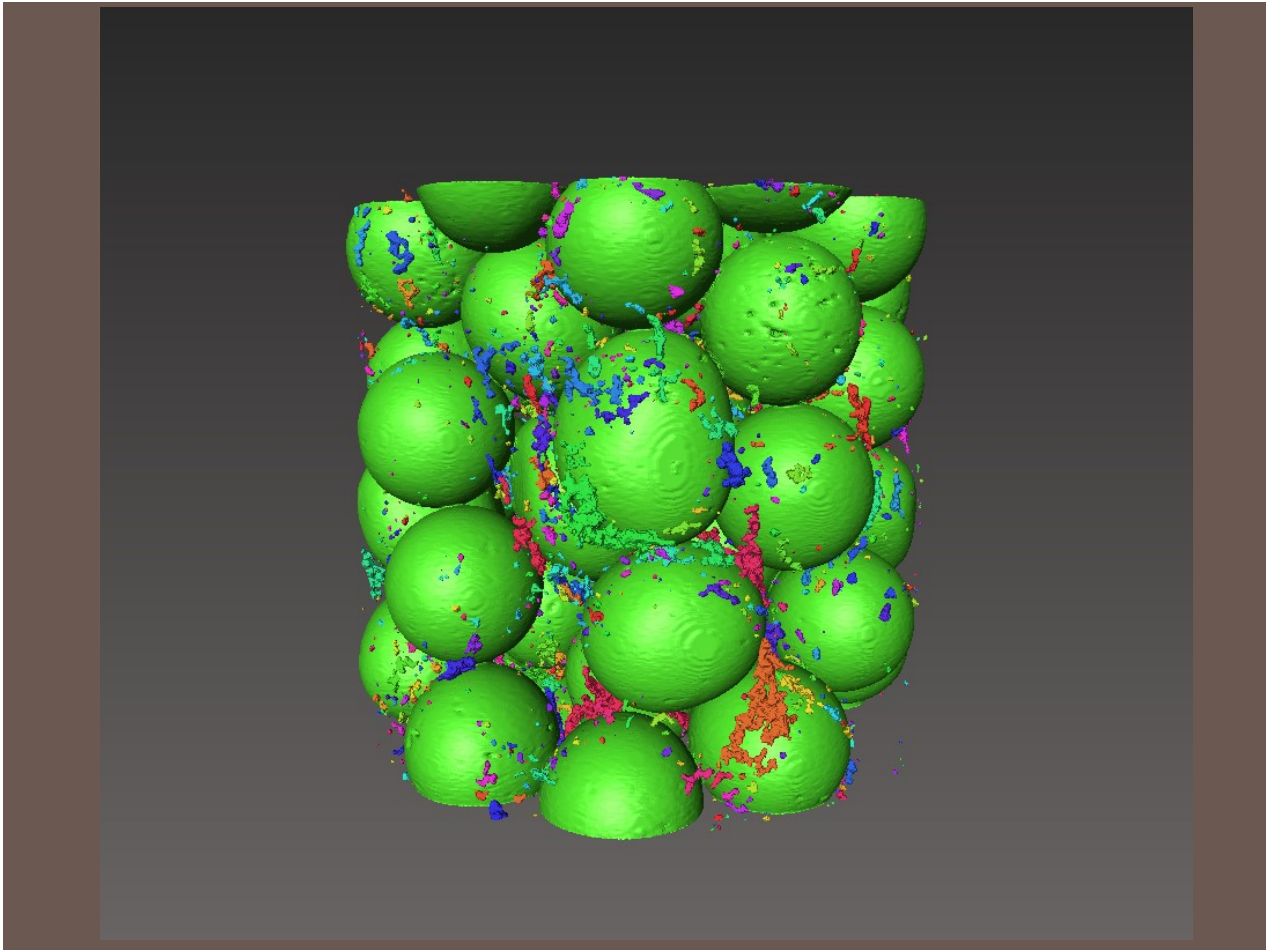
Barium sulfate

ated microspheres





Flow Direction



Contrast Agent Advantages and Limitations

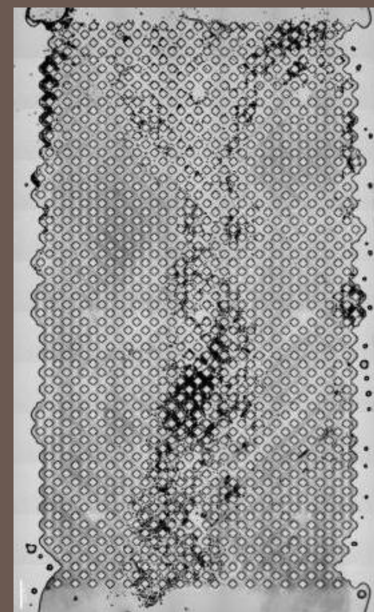
(Silver) Microspheres

- Advantages:
  - Microspheres (particles) can be added to the influent flow stream
  - Surface attachment has occurred for all the biofilms tested so far
- Disadvantages:
  - Biofilm resolution is limited to the mean diameter of the particles
  - Visualization/quantification is directly dependent on the quality of particle coverage on the biofilm

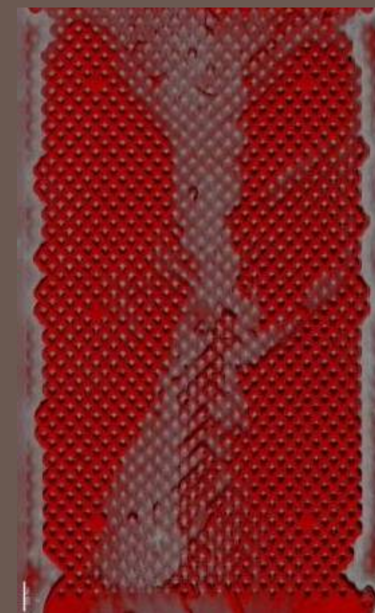
(Barium Sulfate) Suspension

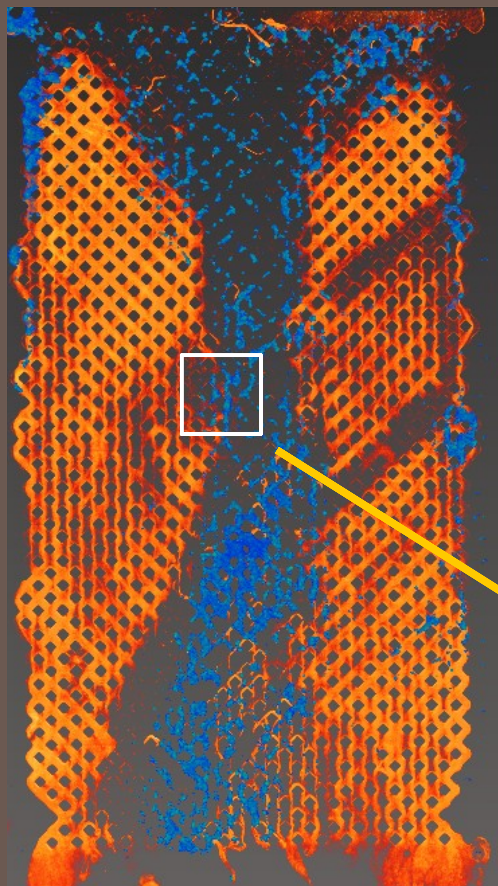
- Advantages:
  - Suspension fills the hydraulically available pore space facilitating "easy" segmentation
  - Good biofilm delineation
  - Particle size is ~ 1 um
- Disadvantages:
  - High density and viscosity requires dilution and special care during addition to prevent dislodging of biofilm
  - Potential compression of low-density biofilm?

Silver particles from CLSM
James Connolly, MSU 2010

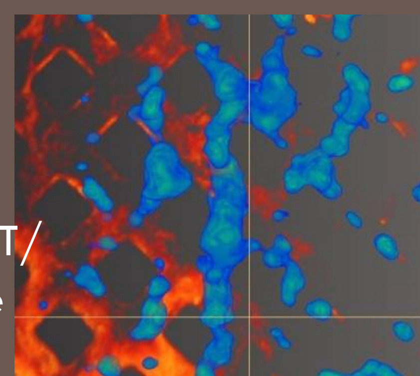


Stained biofilm from CLSM
James Connolly, MSU 2010



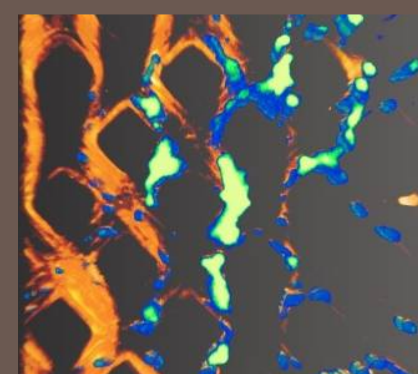


Silver microspheres from CMT overlain on rhodamine stained biofilm from CLSM



Zoomed CMT/ CSLM image

CLSM image



Quantitative Studies

- Effect of flow rate on biofilm growth and porosity change
- Effect of bacterial species
- Measure pressure changes, oxygen concentrations etc.
-

  – see poster

Next!

- Combined quantification of biofilms and precipitate for SBR project on Precipitation at Solution-Solution Mixing Zones in Porous Media (Rick Colwell, PI)

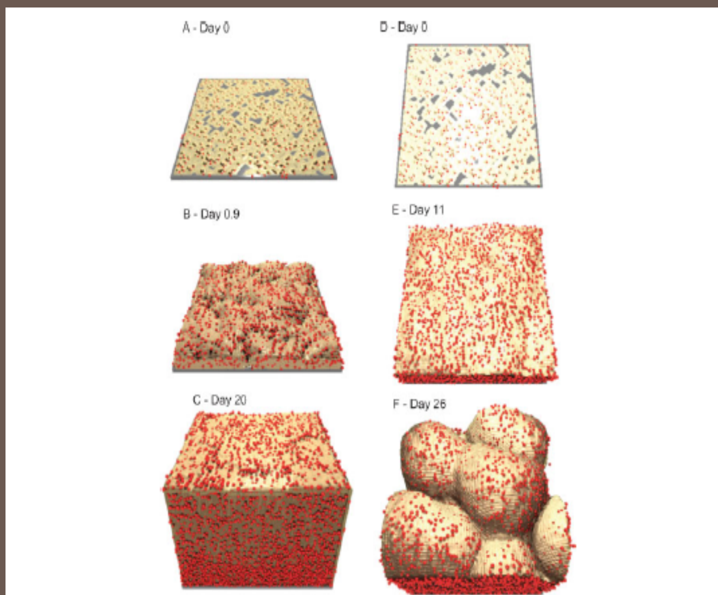
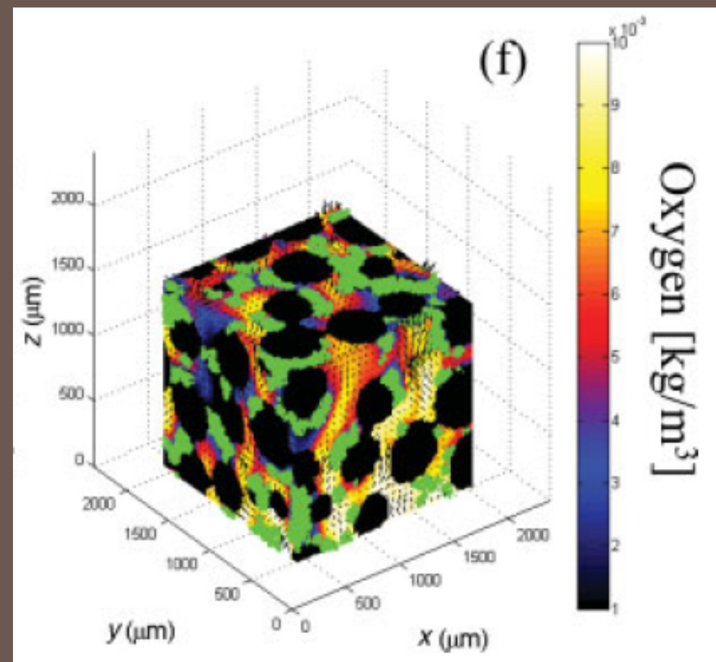


Figure 7. Examples of 3-d biofilm development simulations: (A–C) case *1A3d* (DO 4 g/m^3 and COD 100 g/m^3 in bulk liquid)—oxygen availability leading to a flat biofilm surface; (D–F) case *1B3d* (DO 0.5 g/m^3 and COD 100 g/m^3 in bulk liquid)—oxygen limitation leading to irregular biofilm surface. Heterotrophic bacteria are illustrated by red spheres and the EPS matrix by a light-yellow partially transparent surface. Biofilm height in panel C is 290 μm and in panel F is 235 μm. A higher density of cells can be seen at the base of the biofilm in panel C.

Alpkvist et al. (2006)



Graf von der Schulenburg et al. (2009)

- Numerical Modeling

Conclusions

- The use of particle- and suspension-based x-ray tomography contrast agents facilitates quantitative imaging of biofilm in three-dimensional opaque porous media
- Architecture and spatial distribution can be obtained over many centimeters, for a variety of bacteria, and in a short time frame
- The technique needs refinement for application under a variety of conditions
- Implications for biofilm modeling:
  - Experimental data can be collected non-destructively for calibrating or validating models incorporating biofilm growth and related impacts on transport pathways/hydrodynamics
  - And: "*provide sufficient scientific understanding such that DOE sites would be able to incorporate coupled physical, chemical and biological processes into decision making for environmental remediation and long-term stewardship*"

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